The Saccharin Controversy

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Saccharin and its salts are the most extensively consumed artificial sweeteners in the United States today. The current controversy about the risks of their use to human health has surfaced from research findings that report an increased incidence of cancer, primarily of the urinary bladder, in certain animal species and man chronically exposed to these agents. The April 1977 proposal by the Food and Drug Administration to restrict use of saccharin was based on these investigations. The intense public response against any ban has led to Congressional deliberations over the fate of saccharin during the present moratorium and information-gathering period. Since diabetic patients are among the principal users of this compound, it appears timely to review the evidence for and against its carcinogenic potential. DIABETES CARE *1*: 211–222, JULY–AUGUST 1978.

In 1879, a Johns Hopkins University team of Remsen and Fahlberg synthesized saccharin from toluene in this country. When the concentrations of the sodium salt and sucrose are equal, the relative sweetness of saccharin is 200 to 700 times greater depending on the concentration of the sucrose standard.[1] This gave rise to its popularity as a sugar substitute particularly during major war efforts when suppliers of natural sweeteners were limited.

Following the Food and Drug Administration (FDA) ban of cyclamates as an artificial sweetener in this country in 1969, saccharin consumption increased substantially. In 1972, 4.2 million pounds were produced or imported of which 3.7 million pounds were used as a food additive in some form. By 1976, this figure was between 6 and 7.6 million pounds, and 70 per cent served as an additive for foods or beverages. Other industrial products include it as an ingredient in cosmetics, pharmaceuticals, and animal feed; more specifically in items ranging from lipsticks, toothpastes, and mouthwashes to skin lotions and creams, colognes, powders, and soaps. Both prescription and nonprescription drugs may contain saccharin, especially those used by pediatric and diabetic patients. Approximately three quarters of all saccharin consumed as a food additive is in the form of beverages. The remainder is contained in "sugar-free" desserts, sauces, fruit preserves, salad dressings, canned fruits, and chewing gum. In 1972, it was estimated that about 50 per cent of citizens in Canada and U.S.A. drink carbonated beverages, and, within this group, 12 per cent drink the low calorie variety. At that time this would represent about 12 million Americans or about 6 per cent of our entire population.[2]

It is estimated that average soft drink consumption is 16.6 ounces per day with a 90th percentile figure of 30 ounces. This statistic can be broken down further into average and maximum consumption by age groups. It has been estimated that the greatest mean daily intake of soft drinks is in the 18- to 24-year-old group: 18.2 ounces with a 90th percentile level of 42.4 ounces. Since a National Academy of Sciences survey determined that maximum concentration of saccharin in such beverages is 0.0287 per cent, this age group theoretically would consume maximally 347 mg. daily. Consumption in other age groups is estimated on a similar basis and is listed in table 1.

METABOLISM

There is little evidence that saccharin is metabolized or stored after ingestion by mammalian species including man. Although earlier isotopic studies with saccharin suggested that some metabolic conversion to O-sulfamoylbenzoic acid or O-sulfobenzoic acid does occur,[3] more recent investigations of rats, guinea pigs, monkeys, and man have failed to

TABLE 1
Estimated maximum daily saccharin consumption from soft drinks in various age groups*

Age group (yrs.)	Daily saccharin consumption (mg.)
6–11	160
12–17	232
18–24	347
25–49	219
Over 50	239

* National Academy of Science GRAS survey work has determined a maximum content of saccharin of 0.0287 per cent in artificially sweetened soft drinks. Ninetieth percentile consumption of soft drinks by age groups is based on a 1972 report of the Beverage Manufacturing Industry.

confirm these observations.[4–6] Byard and Goldberg,[4] as well as Minegishi and co-workers,[5] reported that highly purified ^{14}C-saccharin administered orally to rats, guinea pigs, or monkeys promptly appeared unchanged in urine. Eighty-five to over 90 per cent of the test dose was eliminated within 48 hours by this route. A small fraction is recovered in feces and only trace amounts in bile.[4] Very similar results have been obtained in adult human subjects whether ^{14}C-labeled or unlabeled saccharin was given.[6]

Of interest is the finding by Pitkin, Reynolds, Filer, and Kling[7] that ^{14}C-saccharin administered intravenously as a 60-minute infusion to Rhesus monkeys in late pregnancy does cross the placenta to a limited extent. Moreover, significant fetal levels were still present five hours after completion of the infusion and two hours after maternal blood contained only trace amounts. The distribution of saccharin to fetal tissues was uniform except for sparing of the central nervous system. These data suggest that clearance of saccharin in the fetus is considerably slower than in the gravid mother and raises the possibility of greater degrees of saccharin accumulation in the fetus with continuing maternal ingestion of the compound.

IMPURITIES IN COMMERCIAL SACCHARIN

Saccharin is manufactured in several different ways. In the Remsen-Fahlberg method, toluene is the starting material; in the Maumee process, it is phthalic anhydride. A variety of impurities may arise from the manufacturing process and apparently vary considerably from one batch or source to the next.

In a recent survey of water-soluble and insoluble contaminants, Stavric reported his analyses of various commercial preparations.[8] The range of contaminants was between 40 and 7,000 p.p.m. The impurities that were specifically identified after bulk extraction included ortho- and para-toluene sulfonamide, 1-2-benzothiazole dioxide, 1-2-benzothiazole, 1-1-diazolene dioxide, diaphen, three different structures of diatole, and para-para-anadine. It is of major interest that some animal studies employed saccharin that was relatively high in content of impurities. This will be discussed in a subsequent section.

CARCINOGENICITY

There are several methods for assessing cancer risks of any food ingredient to which man is exposed. Saccharin has been investigated extensively in several human case studies involving primarily bladder cancer. Several prospective studies of animals on fixed doses of this agent have been reported. In addition, mutagenicity testing of saccharin in a variety of in vitro and in vivo systems has been performed. At the present time each method has served to link saccharin consumption to cancer in at least one study while others employing similar methods have failed to corroborate this finding. The lack of agreement among scientists utilizing the same general techniques has generated the controversy over saccharin as it exists today.

MUTAGENICITY STUDIES

Various techniques have been developed to detect mutagenic effects of chemical substances, including saccharin. These include mutation frequencies of microorganisms and genetic damage or aberrations sustained in larvae, gametes, plant seeds, human and animal leukocytes, and other animal tissue cells after direct exposure to potential carcinogens in vitro. Others examine the effects of a test substance on mammalian tissue cells after its administration to the intact animal. The rationale for this line of investigation is that carcinogens generally are mutagens. In one series employing the sensitive in vitro technique of Ames and co-workers, up to 85 per cent of known carcinogens had demonstrable mutagenic effects on selected strains of bacteria.[9] This supports the view that carcinogens cause cancer by interaction with cellular genetic components.

Studies of saccharin with these methods have uncovered no clear-cut information about its carcinogenic potency. In Kramers' review,[10] seventeen investigations of this type were summarized through 1973. He concluded that in some reports saccharin was weakly mutagenic in certain bacteria, in the fruit fly, and in mice with moderate to very high doses of the sweetener. It also appeared to break chromosomes in the onion root tip and Chinese hamster cells. However, there were a comparable number of negative results emanating from other laboratories in which similar or different techniques were employed. Collectively, the investigations were "too conflicting and equivocal" to regard saccharin as a proven mutagen. He also suggested that inconsistent results

may relate to varying amounts of mutagenic impurities in various preparations of saccharin rather than to saccharin itself.

The problem of impurities arose once more in very recent studies of Stoltz and co-workers.[11] They showed that impurities in commercial saccharin produced by the Remsen-Fahlberg or Maumee processes are mutagenic utilizing the Ames procedure.[9] Subsequently, Batzinger, Ou, and Bueding[12] performed similar tests on six lots of saccharin: A, material obtained from a local pharmacy; B, a sample manufactured by the Sherwin-Williams Co.; C, lot S 1022 saccharin employed by Stoltz in his studies cited above; D, highly purified saccharin prepared from lot S 1022 by Stoltz; E, a commercial table sweetener in powder form containing 40 mg. of sweetener and 1 gm. of filler per packet; and F, another saccharin preparation from Stoltz, lot S 1233.

Three lines of experimentation were done in which modified Ames testing was performed on two strains of Salmonella, TA 100 and TA 98. Direct in vitro incubations revealed that only highly purified saccharin (preparation D) failed to have significant mutagenic activity. The variable patterns of mutation in the bacterial strains with other forms of saccharin suggested that more than one contaminant was present. Similar results were obtained when saccharin in the form of A, B, C, and D was fed to mice and either the urine was tested for mutagenicity or bacteria, injected intraperitoneally, were examined for mutation frequency during the feeding experiments. The purified form D was without significant effects in almost all instances in contrast to the other types.

When the Office of Technology Assessment (OTA) received the directive from Congress in 1977 to evaluate the saccharin question, it commissioned twelve reputable laboratories to perform short-term mutagenicity studies on saccharin. As of October 1977, 10 had been completed and the results were published.[13] Each of the 10 utilized a different testing procedure. Table 2 lists the various tests, investigators, and results. Seven studies were negative including the well validated Salmonella/Ames procedure carried

TABLE 2
Short-term mutagenicity testing of saccharin commissioned by OTA*

Investigators	Location	Method	Result
S. Wolff, B. Rodin	Laboratory of Radiobiology University of California, San Francisco	Sister chromatid exchange	Positive
D. Clive	Genetic Toxicology Laboratory Burroughs Wellcome Co. Research Triangle Park, N. C.	Mouse lymphoma	Positive
A. Hsie, J. San Sebastian	Oak Ridge National Laboratory Oak Ridge, Tenn.	Chromosome aberration	Positive
B. Ames, J. McCann	Dept. of Biochemistry University of California, Berkeley	Salmonella/Ames	Negative
V. Simmon	Stanford Research Institute Menlo Park, Calif.	Mitotic recombination in yeast	Negative
H. Rosenkranz	Dept. of Microbiology New York Medical College Valhalla, N. Y.	Pol test (*E. coli*)	Negative
S. Abrahamsen, R. Valencia	Dept. of Zoology University of Wisconsin Madison, Wis.	Drosophila (sex-linked recessive lethal test)	Negative
H. Stick	Cancer Research Center University of British Columbia Vancouver, Canada	Unscheduled DNA synthesis (human fibroblasts)	Negative
R. Pienta	Frederick Cancer Research Center Frederick, Md.	In vitro transformation (hamster embryo cells)	Negative
I. Weinstein	Institute of Cancer Research Columbia University New York, N. Y.	Induction of plasminogen activator (Hela cells)	Negative

* Report of the Office of Technology Assessment to the Committee on Human Resources, United States Senate, October 1977.

out by Dr. Ames' group. Three studies were positive, but only at high saccharin concentrations, and, in some, a dose-response curve could not be established. It was also admitted that the relevance of mutagenicity to carcinogenicity in some tests had not been clearly documented as yet. Moreover, the OTA pointed out that it was unexplainable why the sister chromatid exchange test and the unscheduled DNA synthesis test gave divergent results. Both were performed on human cells in culture over a similar dosage range, and the latter procedure is a sensitive measure of genetic changes that are likely to be detected by sister chromatid exchange. Nevertheless, one was positive and one was negative (table 2).

The OTA concluded that the results were still consistent with the premise that saccharin possesses weak carcinogenic potential. Moreover, the saccharin used in each experiment was the same as that employed in the 1977 Canadian, two-generation rat study in which second generation male rats had an increased incidence of urinary bladder carcinoma when fed 5 per cent saccharin in the diet.[14] This preparation is relatively pure (20 p.p.m. of impurities) as compared with many commercial preparations (often in the range of 5,000 p.p.m.). The possibility was not excluded that these impurities might still contribute to the positive results.

AN APPRAISAL OF MUTAGENICITY STUDIES

Techniques of this type ultimately will provide a valuable method for testing inherent carcinogenicity of many substances. The Salmonella/Ames test has achieved stature in this regard,[9] and many others undoubtedly will as well. One major conclusion to be drawn at this time is that saccharin is still not a proven mutagen as witnessed by the highly variable test results in highly respected laboratories. Such testing should continue along the lines of ascertaining which one is the best index of true carcinogenicity with the long-term goal of selecting that method which most consistently detects agents with low potency for inducing neoplasia. A second conclusion that is implicit in all of these investigations is that certain impurities derived from the saccharin manufacturing process may be more cancer-inducing than saccharin itself. To date many of these impurities in each test material have not been identified. Until they are, the high-dose saccharin studies that report an increased incidence of bladder carcinoma in rats are still open to question. Resolution of these conflicting reports cannot be achieved until mutagenicity testing procedures are well standardized and the purity of saccharin is assured.

HUMAN STUDIES: NEGATIVE FINDINGS

There are several epidemiologic studies that have explored the possible relationship between saccharin usage and bladder cancer (table 3). All but one have failed to show an association.

Armstrong and Doll[15] performed an analysis of 18,733 patients dying from bladder cancer in England and Wales between 1966 and 1972 and compared them with 19,709 cases in whom death was due to other types of malignancies. Specifically, the percentage of patients with diabetes mellitus in both groups was sought. They determined that the relative risk of bladder cancer in patients with diabetes mellitus, regardless of duration of diabetes, was no different from the nondiabetic population. From other large surveys it was found that consumption of the sweetener was significantly greater among diabetic men and women, as expected, than among nondiabetic individuals of both sexes. In certain subgroups, saccharin was used for more than 20 years.

In a related investigation, Armstrong and co-workers prospectively examined mortality statistics of 5,971 members of the British Diabetic Association over a five-to-eight year period. Again, above-average saccharin consumption in this organization was documented. Total cancer deaths and death due to bladder neoplasms did not exceed the expected mortality of a general population.[16]

In the United States, Kessler did not uncover any statistical evidence of increased cancer mortality, other than carcinoma of the pancreas, in 21,447 diabetic subjects during a 26-year period ending in 1956. Saccharin exposure, however, was not ascertained.[17] In a smaller case-control study by Kessler, 209 cases of recently diagnosed bladder cancer were compared with 209 cases without bladder disease. The two groups were matched in age, sex, race, marital status, and location of recent hospitalizations. Saccharin intake did not differ between the two groups, and the results were unaffected by case-control differences in smoking habits or the presence of diabetes.[18] Three other case-control, retrospective analyses by Morgan and Jain,[19] Simon, Yen, and Cole,[20] and Wynder and Goldsmith[21] also could not establish a statistical relationship between consumption of saccharin and urinary bladder malignancies. These three case-control investigations together with the report by Kessler[18] involved over 700 individuals with bladder cancer and more than 900 control subjects.

Since the frequency of this lesion is considerably increased by cigarette smoking,[21] others have examined the influence of saccharin ingestion superimposed on smoking of cigarettes as an added risk factor in bladder carcinoma. No evidence for this was found. It was emphasized that trends in bladder cancer mortality in smoking male cohorts from 1911 through 1965 have not been altered by the consumption of saccharin between 1939 and 1965.[22]

It is interesting that among the epidemiologic studies cited above that found no association between saccharin usage and bladder cancer, three studies found some association between coffee drinking and this malignancy.[19–21] Although the association was weak as compared with cigarette smoking, the statistical implication was stronger

TABLE 3
Human epidemiologic studies of artificial sweetener consumption and bladder cancer risk*

Investigators	Type of study	Number of subjects	Statistical relationship between artificial sweeteners and bladder cancer	Comments
Kessler[18]	Retrospective case-control, hospital based (1970)	209 cases, 209 controls	None	Association not affected after adjusting for cigarette smoking and saccharin use by diabetics.
Morgan and Jain[19]	Retrospective case-control, hospital based (1974)	158 male cases, 158 male controls, 74 female controls	None	Data on usage of sugar substitutes, soft drinks, and diet desserts analyzed separately and together, with no association found either way.
Simon, Yen, and Cole[20]	Retrospective, case-control, hospital based (1975)	135 white female cases, 390 white female controls	None	No association found for usage of either cyclamates or saccharin.
Wynder and Goldsmith[21]	Retrospective, case-control (1977)	132 male cases, 124 male controls, 31 female cases, 29 female controls	None	10% of male cases and 12% of female cases had ever used artificial sweeteners. Maximum relative risk for use was 0.9.
Howe and co-workers[23]	Retrospective, case-control (1977)	480 male cases, 480 male controls, 152 female cases, 152 female controls	1.6 risk ratio for men using saccharin vs. men never using this sweetener. No risk for women.	Editorial in *Lancet*[24] criticizes methods and analyses.
Burbank and Fraumeni[26]	Population based retrospective analysis of U. S. mortality trends vs. artificial sweetener consumption (1970)	Mortality statistics in the U. S. A. and Connecticut	None	A marked increase in the consumption of artificial sweeteners, chiefly in the form of a 10:1 cyclamate-saccharin mixture, occurred in 1962. No change in the bladder cancer mortality figures occurred in subsequent years, either in Connecticut records or in U. S.-based data.
Kessler[17]	Prospective studies of mortality in diabetics (1970)	21,447 diabetic subjects	None	Series followed from 1930 through 1959. No increased bladder cancer mortality.
Armstrong and Doll[22]	Cohort analysis (1974)	15 cohorts in a population-based series from 1911 to 1970	None	No evidence of any break in continuity of bladder cancer mortality trends that could be attributed to use of saccharin since 1939.
Armstrong and co-workers[16]	Prospective studies of mortality in diabetics (1976)	5,971 members of the British Diabetic Association	None	In a diabetic population (British Diabetic Association) of 5,971, mortality from cancer (8-year period) was less than in a standard population, i.e., 128 were observed and 168 would be expected.
Armstrong and Doll[15]	Death certificate analysis of bladder cancer patients with and without diabetes (1975)	18,733 cases, 18,709 controls	None	Patients with history of diabetes were analyzed. No increased risk for bladder cancer was found in this group of prevalent saccharin users.

* Modified from Wynder, E. L., and Weisburger, J. H.: Editorial: Recommendations for modifying federal statutes relating to environmental carcinogens. Prev. Med. 6: 2, 1977.

than the case for saccharin. In Wynder and Goldsmith's comprehensive study and review of the epidemiology of bladder carcinoma, cigarette smoking and industrial exposure to certain carcinogens were the primary etiologic factors in this disease. However, these factors could not account for all cases of bladder carcinoma. It was suggested that nutrition per se, particularly high dietary intakes of protein, fat, and/or cholesterol may be of etiologic significance.[21]

HUMAN STUDIES: POSITIVE FINDINGS

While most human studies fail to associate saccharin usage with bladder malignancy, one investigation recently published by Canadian researchers, Howe and co-workers,[23] has linked the two together. In their case-control study, 480 men and 152 women with recently diagnosed carcinoma of the urinary bladder who lived in three Canadian provinces answered a comprehensive questionnaire. Control subjects consisted of age- and sex-matched citizens living in the same locations without the malignancy. Apart from general questions concerned with medical history, occupation, drug usage, beverage and smoking habits, nutritional history, etc., specific questions focused on consumption of sugar substitutes, primarily saccharin.

The results of this investigation pointed to a significant risk ratio of 1.6 for males who consumed saccharin versus males who never used the sweetener. This was highly significant statistically. If the data were further analyzed for men with and without diabetes mellitus, the ratios were 1.9 and 1.7, respectively. Calculations of population attributable risks for all men were 7 per cent, and for diabetic men 33 per cent, because of the latter group's greater intake of saccharin. Women were not found to be at risk for bladder cancer within the range of saccharin intake defined.

AN APPRAISAL OF HUMAN STUDIES

The great majority of human epidemiologic studies of the relationship between the use of saccharin and urinary bladder cancer have been negative to date. Although this is comforting, two opposing conclusions can still be drawn from these results: that there is no relationship between the two, or, and most important, that the carcinogenicity of this sweetener is weak and cannot be detected by conventional studies of a design such as these. The basis for the second conclusion is summarized by the FDA itself.

In the Federal Register of April 4, 1977, the FDA's reasons for proposing a saccharin ban dealt, in part, with published human studies. In their opinion such investigations cannot detect a carcinogenic risk unless it exceeds a control population by at least two- to three-fold. By assuming that animal studies of saccharin can be extrapolated to the human situation, they further estimated that life-long consumption of 150 mg. of this sweetener daily by the entire American public (equivalent to one large diet drink daily) would increase the current incidence of bladder cancer by only 4 per cent. Furthermore, they point out that significant consumption of this agent has not been long enough to draw any meaningful conclusions about a potentially weak carcinogen by such investigations. In this instance 25 to 30 years of exposure in a very large population may be required.

If one accepts the fact that negative epidemiologic studies do not necessarily exclude weak or moderate carcinogenicity of a material like saccharin, it is also disturbing that one report by Howe and colleagues supports a cancer risk.[23] However, in an editorial accompanying this publication in *Lancet*, several criticisms were levied against the validity of this report.[24]

In the editorial the strongest objection to the study was the finding of sex differences in the relationship between saccharin consumption and bladder cancer. Women using saccharin were found to have a slightly lower incidence of the carcinoma than female control subjects and only men ingesting this agent were at risk. This, by itself, lacks credibility according to the editorial, since most "epidemiologists are familiar with the dangers (and temptations) of drawing conclusions from subgroups when the total results (i.e., both sexes combined) are not significantly different from expected." The editorial goes on to state, "No nonhormonal carcinogen in man is known to affect only one sex, and the case made by the Canadian workers that saccharin is the first such example is less than convincing." Why this case-control study was positive for one sex whereas similarly designed investigations were not (table 3) remains unexplained. The editorial implies that critical factors like cigarette and coffee consumption were not adequately quantitated to remove the possibility of bias in the various groups. Selection of control subjects was also criticized. Similar conclusions were reached by Dr. Philip Cole of the Harvard School of Public Health in his critique of the manuscript before its publication.[25]

ANIMAL STUDIES

Prospective studies of the saccharin-cancer relationship in animals have been designed in at least three different ways and largely confined to mice, rats, guinea pigs, or monkeys: (1) Single generation feeding investigations in which the per cent saccharin in the diet ranges between 0 and 7.5 per cent (A 5.0 per cent diet of saccharin is approximately 2.5 gm. per kilogram body weight per day and would be equivalent to about 800 diet soft drinks containing saccharin per day.) (2) Two-generation studies in which parents are fed in a similar fashion (which includes the period of pregnancy and weaning in females) and in which continuation of the same saccharin regimen is begun in the offspring as in the parents and is continued throughout the lifetime of the second generation.

(3) Co-carcinogen studies that test the influence of saccharin coingested with known cancer-causing agents.

There are seven single generation rat studies now reported.[27–33] Three have been published in recognized journals,[27,32,33] and, of these, one has been detailed in its findings.[33] All have failed to show any relationship between bladder cancer and saccharin ingestion up to dosage levels of 7.5 per cent and through periods of 24 months or more. In some reports the search for bladder parasites and calculi have been sufficient to exclude these as etiologic factors or modifiers of the incidence in control versus saccharin-fed groups.[33] Both the National Academy of Science[34] and the recent report of the Office of Technology Assessment[13] have accepted the conclusions of these reports. Similar results have been found in mice[31,35,36] and in hamsters,[37] although some were difficult to interpret conclusively as reviewed elsewhere.[13]

In studies of monkeys, McChesney, Coulston, and coworkers report the effects of oral saccharin on Rhesus monkeys over a period of 6.7 years.[38,39] Dosages were 0 (control), 20, 100, and 500 mg. per kilogram body weight per day. No mortality was attributable to the agent. During life there were no abnormalities detected in general health or in blood and urine elements. At autopsy, studies of all organs grossly were negative as were microscopic studies of liver, kidneys, testes, and urinary bladders. These results in nonhuman primates support the negative observations reported in rodents.

Two-generation rat studies are of particular interest, since they formed the basis for the proposed ban of saccharin by the FDA. There were three investigations performed, and each found a significant increase in bladder cancer, mostly in male rats at high-dosage levels in the second generation. Table 4 summarizes the results of these reports.

The first two studies were conducted by the FDA and WARF (Wisconsin Alumni Research Foundation) and were published in 1973.[40,41] Neither investigation collected histologic data on first generation rats. The Canadian study, as of December 1977, has not been published but details of this work are available.[14] Data include those derived from the first generation. The WARF study is incomplete in that histological studies were performed on most, but not all, second generation rats.

The three investigations had some common findings: (1) Saccharin at all dosage levels did not increase mortality. (2) Between the 5.0 and 7.5 per cent dose, carcinoma of the urinary bladder was significantly greater in second generation male rats. Between 4 and 6 per cent of the second generation female rats developed the lesion with similar doses in the FDA and Canadian studies. In addition, first generation male rats had a higher incidence of bladder cancer (8 per cent) at 5 per cent doses of saccharin in the Canadian study. Moreover, there was a higher incidence of urinary bladder hyperplasia reported in the FDA investigation with 7.5 per cent saccharin intakes. (3) Parasites and urinary calculi were satisfactorily excluded as etiologic factors. (4) No dose-response curve could be constructed from the data when considered separately or collectively. (5) Signs of toxicity were present in each at 5 or 7.5 dosages, since weights after weaning and adult weights of the second generation were 10 per cent or more below control weights during most of the exposure period in the FDA and Canadian studies. For all three investigations a slower rate of weight gain was observed. Final weights were less affected in male as opposed to female rats.[13]

TABLE 4
Incidence of rat bladder carcinoma in the second generation of two-generation feeding experiments*

Dose (%)	Number of carcinomas/total examined with per cent incidence (in parentheses)		
	FDA, 1973	WARF, 1973	Canadian, 1977
Male rats:			
0	1/25 (4)	0/12 (0)	0/42 (0)
0.01	0/16 (0)	—	—
0.05	—	0/10 (0)	—
0.10	0/27 (0)	—	—
0.50	—	0/12 (0)	—
1.0	0/22 (0)	—	—
5.0	1/21 (5)	7/15 (47)	8/45 (18)
7.5	6/23 (23)	—	—
Female rats:			
0	0/24 (0)	0/16 (0)	0/47 (0)
0.01	0/23 (0)	—	—
0.05	—	1/14 (7)	—
0.1	0/24 (0)	—	—
0.5	—	0/15 (0)	—
1.0	0/30 (0)	—	—
5.0	0/28 (0)	0/20 (0)	2/49 (4)
7.5	2/31 (6)	—	—

* Duration of exposure to saccharin approximated 18 to 25 months in the three studies.

In the report by the Office of Technology Assessment,[13] it was also accepted that the Canadian study had satisfactorily excluded ortho-toluene sulfonamide (OTS) as a carcinogenic agent. This material was fed to other groups of rats to distinguish its role as a known saccharin contaminant in the genesis of neoplasia. The results were negative.

At this point it also should be mentioned that neoplasms other than urinary bladder carcinomas have been studied in many of the single and two-generation studies. These included a variety of soft tissue carcinomas and/or sarcomas. This subject has been extensively reviewed by Reuben[42] who associated some of these lesions with saccharin exposure. However, in the opinion of OTA, the spontaneity of these malignancies in control animals, the lack of consistent

saccharin dose-response-tumor incidence, the small number of animals, and the variability of study techniques and emphasis precluded any justification of a cause-and-effect relationship between saccharin and nonbladder tumors.[13]

CO-CARCINOGEN STUDIES

One method of assessing cancer-promoting properties of saccharin is to administer it separately or combined with a known carcinogen. Two studies by Hicks were reported in 1973 and 1975.[43,44] The basic design consisted of groups receiving no treatment, a 4 to 8 per cent saccharin diet, a single bladder instillation of a known bladder carcinogen, *N*-methyl-*N*-nitrosourea (MNU), or saccharin plus MNU. MNU in a single 2 mg. dose does not produce bladder neoplasms, and a negative result was found as in the control group. In the 1975 study saccharin alone induced bladder tumors in only 4 out of 253 animals (1.6 per cent) after the 95th week of exposure. However, MNU plus saccharin produced bladder tumors in 46 of 79 rats (58 per cent) and the first lesions were observed as early as eight weeks. The actual microscopic histology of these tumors has not yet been reported. In the 1973 investigation only small numbers, 12 in each of the four groups, were killed at 3 to 56 weeks. Bladder epithelial hyperplasia was noted in a small number of saccharin-only or MNU-only treatment groups. With the combined regimen 5 out of 12 had bladder tumors of which 3 were carcinoma, and 6 out of 12 had hyperplasia. The results led the authors to conclude that saccharin has co-carcinogenic potential.

Quite paradoxically, Ershoff and Bajwa[45] performed similar studies on female rats fed daily with 300 mg. of 2-acetylaminofluorene (AAF) separately or combined with 5 per cent saccharin or 5 per cent cyclamate. Both nonnutritive sweeteners reduced ear duct and mammary gland tumor incidence from 92 per cent with AAF alone to 17 per cent (cyclamates + AAF) or 50 per cent (saccharin + AAF). Both sweeteners also reduced the size and severity of liver carcinomas and did not hasten the development of urinary bladder tumors, since none was found in control or treatment groups up to 40 weeks of this study. These authors concluded that carcinogenicity of AAF with respect to ear duct, mammary gland, and liver tumors is suppressed by saccharin or cyclamate administration and that neither sweetener potentiates the development of urinary bladder tumors by AAF in the doses employed.

AN APPRAISAL OF ANIMAL STUDIES

The prevailing philosophy for testing agents for carcinogenesis in laboratory animals has been adequately and responsibly defined by the National Cancer Institute[46] with additional recommendations by the National Academy of Sciences.[47] These guidelines include the following:

(1) Testing of 100 animals, 50 male and 50 female of two species, which is to begin within six weeks of birth and extended for a period that is long enough to produce a maximum response (usually 24 months). The same number of control animals is included. In addition, the NAS has recommended that two-generation studies be considered to allow in utero exposure to the test agent in the second generation.

(2) Administration of the test substance by the same route as that taken by humans. At least two doses should be given: a maximum tolerated dose and fractions thereof (½, ¼, etc.). The maximum dose should not induce signs of toxicity nor increase mortality.

(3) Termination of experiments should be followed by adequate tissue examinations.

The FDA has condoned short-term, high-dose testing experiments in rats with the understanding that meaningful dose-response curves can be extrapolated to long-term, low-dose exposure in humans. Mathematical extrapolations of this type have been reviewed elsewhere.[13] The basis for this kind of approach is implicit in the NCI, NAS recommendations enumerated above and can be defended by experiences with known carcinogens.

Probably the one investigation that comes closest to satisfying these experimental guidelines is the one generation study of Munro[33] in which adequate numbers of animals were tested and detailed studies were described and published in a refereed journal readily available to the scientific community. The results along with other one generation studies were clearly negative. Although the prospective investigation of Rhesus monkeys suffers from a small number of animals, the use of this nonhuman primate for cancer testing has been justified by others.[48] It is noteworthy that after nearly seven years of high-dose saccharin exposure no histologic changes of any type were observed in urinary bladders of this animal species.[38,39]

The most alarming finding in this work centers on two-generation rat studies (table 4). It is quite clear that all three investigations have uncovered a significant induction of bladder tumors in second generation rats exposed to dietary saccharin doses of 5 or 7.5 per cent. The OTA has accepted these data as supportive of saccharin's carcinogenicity.[13] In this light, one generation studies are to be regarded as inconclusive rather than negative, since the two-generation model is a more sensitive test involving in utero as well as adult exposure.

However, there are certain inherent weaknesses in the two-generation feeding experiments. First, the data reported in table 4 do not allow one to construct a meaningful dose-response curve. All saccharin intakes below the 5 per cent level are without carcinogenic effect. Thus, a threshold for the induction of bladder carcinoma has not been defined. This is in marked contrast to dose-response relationships between cigarette smoking and this bladder neoplasm.[21]

A second major criticism of the two-generation experiments is that carcinogenicity may be associated with signs of toxicity. In these instances second generation (F1) offspring of 5 or 7.5 per cent saccharin maternal groups were more than 10 per cent below weights of control offspring at the start of experiments. They continued to carry subnormal weights and/or gain weight subnormally throughout most of the 5 and 7.5 per cent saccharin exposure in the FDA and Canadian studies. According to strict NCI guidelines, carcinogenicity data are subject to question when attended by toxicity.[46] In other words, deviations from normal weight patterns may indicate that toxicity was present, but how this relates to carcinogenesis in these studies remains to be determined.

Recall that saccharin crossing the placenta is cleared more slowly by the fetus than by the adult.[7] It is possible that unusually high levels may have been achieved in the developing embryos of the F1 generation. The nature of any adverse effect is unknown, because no chemical or histological examinations were performed on these animals at birth or after weaning. One could speculate that saccharin exposure, if overwhelming in utero and during weaning, could result in extremely high urinary concentrations which may alter the natural protective integrity of the developing bladder mucosa and increase its vulnerability to otherwise insignificant bladder carcinogens in later life. Such cancer-inducing factors could reside in saccharin contaminants, arise from interactions of saccharin with food, or be present in the cage environment. Some have suggested a need for testing of the latter two possibilities.

While it is true that saccharin-fed, F1 generation female rats demonstrated greater weight deviations from control animals but had a lower incidence of bladder carcinoma as compared with saccharin-fed males, this does not exclude toxic side effects, particularly on the urinary bladder, as important conditioning events. The fact that two-generation, high-dose feeding experiments were more devastating for male animals is of interest since Howe and co-workers also emphasized a male sex predilection in their human epidemiological study of saccharin.[23] However, for reasons discussed earlier, a nonhormonal carcinogen should not favor one sex over another unless more than one contributing factor is involved.

A third criticism of these studies is the lack of accountability for potentially carcinogenic saccharin contaminants. According to Stavric[8] and Stoltz,[11] saccharin used in the 1973 FDA and WARF studies, as well as in the co-carcinogen study of Hicks,[43,44] may have had substantial levels of impurities.[8] We have already discussed earlier that some of these contaminants are probably mutagenic.[11,12] The possibility exists that these may have had an etiologic role in bladder cancer induction.

On the other hand, saccharin used in the 1977 Canadian study was relatively pure (20 p.p.m.), but the 5 per cent dietary dose still increased bladder carcinoma incidence in second generation male rats.[14] If saccharin were directly carcinogenic, as opposed to other factors just cited, it remains inexplicable why this same material was not mutagenic in the majority of OTA-commissioned laboratories, in particular with Salmonella/Ames testing by Dr. Ames' group (table 2). It is generally agreed that this particular test is very sensitive and, when positive, is predictive of carcinogenic potential.[9] These conflicting data also strengthen the view that saccharin may not be the primary agent responsible for bladder neoplasia in the three studies.

SACCHARIN: RISK VERSUS BENEFIT

Sugar as a nutritive sweetener has become an integral part of the world diet. After its introduction to Western civilization from the near East during the crusades, the demand for it became intense even though at first it was a luxury. General consumption became significant only after the industrial revolution. In 1900, world production of sugar was 8 million metric tons. By 1970, this had risen to about 70 million. The Food and Agricultural Organization of the United Nations predicts a figure of 93 million tons by 1980. The rate of rise in production is exceeding the rate of rise in world population.

In the United States and the United Kingdom per capita consumption has averaged 50 to 60 kg. per year for several years. In America this would represent about 0.3 pound per person per day of sugar in some form or another.

The demand for foods flavored with concentrated nutritive sugars surrounds the life style of those individuals who must restrict them in order to control diabetes, obesity, or unusual degrees of tooth decay. Without question the availability of artificial sweeteners has improved their quality of living. Since the number of Americans with diabetes and/or obesity approaches 20 per cent of our nation's population and the number with an increased predilection for tooth decay is 10 per cent, it is not surprising that the FDA proposal of April 1977 to ban saccharin was met with fervent public opposition. Congressional mail was voluminous and averaged 200 to 1 against the proposal according to some sources.

Nevertheless, there is no sound scientific evidence that nonnutritive sugar substitutes have greatly improved the ease of management of these patients even though opinions of the medical and dental professions appear to favor the continued availability of these agents. Moreover, there is no scientific information available to tell us what the impact of saccharin withdrawal would be on these groups of patients. Peer pressure, particularly on young adults who consume the largest quantities of soft drinks, could lead segments of these groups to digress more readily from prescribed dietary regimens. Whether this would result in more difficult care and acceleration of complications of these disorders is not known.

A survey of individuals with diabetes mellitus reports that if saccharin were banned, over 80 per cent would revert to using nutritive sweeteners to satisfy their desire for foods and beverages with a sweet taste.[49] This raises an important question about the relative risk and/or feasibility of including sucrose, fructose, sorbitol, xylitol, and similar sugars in diabetic diets to a much greater extent if no nonnutritive sweetening agents like saccharin were available. This poses a problem which must take into consideration the extra carbohydrate calories such nutritive sugars would add to the diet; the degree to which each sugar would influence diabetic control adversely; and possible long-term effects of these different carbohydrates on complications of the disease. Would their increased consumption create more health problems than the present use of saccharin?

Although one cannot define the benefits of nonnutritive sweeteners with certainty, one can place the risk of using saccharin in some perspective. If bladder cancer is the disease in question, cigarette smoking is a definite proven etiologic factor as are certain industrial exposures. Human epidemiologic studies also find an association with coffee consumption, but the trend or association is not significant.[19–21] These same investigations find no association between saccharin consumption and the lesion. Thus, it appears that saccharin-containing foods or beverages are clearly the lowest on a continuum of risk factors for bladder cancer among three popular habits in this country: cigarette smoking, coffee drinking, and nonnutritive sweetener usage. Because saccharin is a food additive and is subject to the Delaney Amendment, whereas cigarettes and coffee are not, the FDA was bound by law to propose the saccharin ban based on two-generation rat studies. The question now is whether the best interests of the public are served by further restricting saccharin when the risk/benefit ratio is not well defined and, if anything, the true risk may be quite low or nonexistent.

SUMMARY

Human epidemiologic studies have failed to uncover a relationship between saccharin consumption and urinary bladder carcinoma in all but one instance (table 3). The one positive retrospective study reported recently[23] has been severely criticized for defects in design and analysis.[24] All studies suffer from lack of sensitivity, however, and may not delineate low grade, weak carcinogens.

Mutagenicity testing of saccharin is very equivocal and probably relates to the variable levels of mutagenic impurities in various preparations of this sweetener. The Salmonella/Ames test, for example, is positive 85 per cent or more of the time when known carcinogens are present. Highly purified saccharin has been negative on two recent occasions[12] (table 2), whereas commercial preparations containing impurities have been positive.[12]

The association between cigarette smoking or certain industrial exposures and bladder cancer is well established. Other associations include coffee consumption and diet, but these associations are not statistically significant. In these same studies saccharin usage did not show any trend or association with this malignant lesion.

Single generation feeding experiments containing dietary saccharin levels of up to 7.5 per cent have not revealed any significant effect of this sweetener on the development of bladder tumors in rats, mice, guinea pigs, and monkeys.

Two-generation feeding experiments have reported an increased incidence of bladder carcinoma in second generation male rats with dietary levels of 5 or 7.5 per cent. However, these experiments may be inconclusive from several standpoints:

(a) Animal numbers are small and a dose-response curve cannot be defined as it often can be when a substance is a true carcinogen.
(b) Five and 7.5 per cent dietary intakes which induced bladder tumors were associated with subnormal weight gains in the F1 generation. This may indicate that generalized toxicity was present during portions of these experiments which is contrary to guidelines for animal testing of carcinogens as published by the National Cancer Institute. If this interpretation is valid, it still does not explain why only male rats were afflicted unless contributing events independent of or in addition to saccharin and toxicity were extant.
(c) Two of the three studies employed saccharin with significant levels of mutagenic impurities. Relatively pure saccharin used in the 1977 Canadian rat study was negative by Salmonella/Ames testing even though rats fed this same material developed bladder carcinoma at a 5 per cent dosage level.

The lack of correlation between the mutagenicity of saccharin and the development of bladder cancer in the second generation male rat exposed to saccharin also suggests that a direct role of this agent in the induction of this malignancy has not been established. However, this does not exclude the possibility that saccharin in these very high dosages may adversely affect the vulnerability of the urinary bladder to carcinogens when exposure includes the embryonic and neonatal periods. The relevance of this latter possibility to the human situation cannot be defined adequately at this time.

CONCLUSIONS

Scientific evidence implicating saccharin as a carcinogenic food additive is inconclusive from the standpoints of human epidemiologic investigations, mutagenicity studies, and animal feeding experiments.

Additional, more comprehensive research is required to resolve the saccharin controversy. Until more specific evi-

dence is uncovered that saccharin promotes urinary bladder cancer or other serious lesions in humans and until the relevance of mutagenicity testing and animal feeding experiments with saccharin to human cancer is more uniformly established, it is difficult to define a cancer risk for humans and to justify placing additional restrictions on saccharin use by the American public at this time.

ADDENDUM

This review covers material available to us through December 1977. Subsequently, the National Academy of Science publicly announced in June 1978 that they would also study the problem of risks, benefits, and the question of malignancy as it relates to saccharin consumption. The National Cancer Institute also indicated at that time that they have begun a large human epidemiologic study of bladder cancer that will gather data from several state and metropolitan regions within the United States. The results of these investigations may provide important new information on the subject.

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REFERENCES

[1] Moskowitz, H. R.: The psychology of sweeteners. *In* Sugars in Nutrition. Sipple, H. L., and McNutt, K. W., Eds. New York, Academic Press, 1974, pp. 38–63.

[2] Subcommittee on Non-nutritive Sweeteners, National Academy of Sciences: Safety of saccharin and sodium saccharin in the human diet. Washington, D. C., 1974, pp. 16–19.

[3] Pitkin, R. M., Anderson, D. W., Reynolds, W., and Filer, L. J., Jr.: Saccharin metabolism in Macaca mullata. Proc. Soc. Exp. Biol. Med. *137:* 803–12, 1971.

[4] Byard, J. L., and Goldberg, L.: The metabolism of saccharin in laboratory animals. Food Cosmet. Toxicol. *11:* 391–402, 1973.

[5] Minegishi, K.-I., Asahina, M., and Yamaha, T.: The metabolism of saccharin and related compounds in rats and guinea pigs. Chem. Pharm. Bull. Tokyo *20:* 1351–58, 1972.

[6] Byard, J. L., McChesney, E. W., Goldberg, L., and Coulston, F.: Excretion and metabolism of saccharin in man. II. Studies with ^{14}C-labelled and unlabelled saccharin. Food Cosmet. Toxicol. *12:* 175–84, 1974.

[7] Pitkin, R. M., Reynolds, W. A., Filer, L. J., and Kling, T. G.: Placental transmission and fetal distribution of saccharin. Am. J. Obstet. Gynecol. *111:* 280–86, 1971.

[8] Stavric, B.: Presentation at the Toxicology Forum on Saccharin, May 9–11, 1977, Omaha, Nebraska, pp. 73–81.

[9] Ames, B. N., McCann, J., and Yamasaki, E.: Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res. *31:* 347–64, 1975.

[10] Kramers, P. G. N.: The mutagenicity of saccharin. Mutat. Res. *32:* 81–92, 1975.

[11] Stoltz, D. R., Stavric, B., Klassen, B., Bendall, R. D., and Craig, J.: Presentation at Toxicology Forum on Saccharin, Omaha, Nebraska, May 9–11, 1977, pp. 101–8.

[12] Batzinger, R. P., Ou, S.-Y.L., and Bueding, E.: Saccharin and other sweeteners: mutagenic properties. Science *198:* 944–46, 1977.

[13] Office of Technology Assessment Report to the Committee on Human Resources of the U. S. Senate. U. S. Government Printing Office, Washington, D. C., October, 1977.

[14] Health Protection Branch, National Health and Welfare Dept., Canada: Toxicity and carcinogenicity study of orthotoluenesulfonamide and saccharin. Project E405/405 E. (Unpublished report as of 12/1/77).

[15] Armstrong, B., and Doll, R.: Bladder cancer mortality in diabetics in relation to saccharin consumption and smoking habits. Br. J. Prev. Soc. Med. *29:* 73–81, 1975.

[16] Armstrong, B., Lea, A. J., Adelstein, A. M., Donovan, J. W., White, G. C., and Ruttle, S.: Cancer mortality and saccharin consumption in diabetics. Br. J. Prev. Soc. Med. *30:* 151–57, 1976.

[17] Kessler, Irving, J.: Cancer mortality among diabetics. J. Natl. Cancer Inst. *44:* 673–86, 1970.

[18] Kessler, I. J.: Non-nutritive sweeteners and human bladder cancer: Preliminary findings. J. Urol. *115:* 143–46, 1976.

[19] Morgan, R. W., and Jain, M. G.: Bladder cancer: smoking, beverages and artificial sweeteners. Can. Med. Assoc. J. *111:* 1067–70, 1974.

[20] Simon, D., Yen, S., and Cole, P.: Coffee drinking and cancer of the lower urinary tract. J. Natl. Cancer Inst. *54:* 587–91, 1975.

[21] Wynder, E. L., and Goldsmith, R.: The epidemiology of bladder cancer—a second look. Cancer *40:* 1246–68, 1977.

[22] Armstrong, B., and Doll, R.: Bladder cancer mortality in England and Wales in relation to cigarette smoking and saccharin consumption. Br. J. Prev. Soc. Med. *28:* 233–40, 1974.

[23] Howe, G. R., Burch, J. D., Miller, A. B., Morrison, B., Gordon, P., Weldon, L., Chambers, L. W., Fodor, G., and Winsor, G. M.: Artificial sweeteners and human bladder cancer. Lancet *2*: 578–81, 1977.

[24] Editorial: Bladder cancer and saccharin. Lancet *2*: 592–93, 1977.

[25] Cole, P.: Memorandum to Calorie Control Council, June 24, 1977.

[26] Burbank, F., and Fraumeni, J. F., Jr.: Synthetic sweetener consumption and bladder cancer trends in the United States. Nature *227*: 297–99, 1970.

[27] Fitzhugh, O. G., Nelson, A. A., and Frawley, J. P.: A comparison of the chronic toxicities of synthetic sweetening agents. J. Am. Pharmacol. Assoc. *40*: 583–86, 1951.

[28] Lessel, B.: Carcinogenic and teratogenic aspects of saccharin. 3rd International Congress of Food Science and Technology, Washington, D. C., 1970.

[29] National Institute of Hygienic Sciences, Tokyo: Chronic toxicity study of sodium saccharin: 28 months feeding in mice. Summaries available at National Academy of Sciences, Washington, D. C.

[30] National Cancer Institute: Carcinogenicity of chemicals present in man's environment—final report. Litton Bionetics, Bethesda, Md., Jan. 31, 1973.

[31] National Cancer Institute: Studies on saccharin and cyclamate —final report. Bioresearch Consultants, Inc., Cambridge, Mass., May 31, 1973.

[32] Schmahl, D.: Lack of carcinogenic effects of cyclamate, cyclohexamylamine and saccharin in rats. Arzneim.-Forsch. *23*: 1466–70, 1973.

[33] Munro, I. C., Moodie, C. A., Krewski, D., and Grice, H. C.: A carcinogenicity study of commercial saccharin in the rat. Toxicol. Appl. Pharmacol. *32*: 513–26, 1975.

[34] National Academy of Sciences: Safety of saccharin and sodium saccharin in the human diet. Publication PB 238-137. Springfield, Va., National Technical Information Service, 1974.

[35] National Institute of Hygienic Sciences, Tokyo: Chronic toxicity study of saccharin: 21 months feeding in mice. Reports available at the National Academy of Sciences, Washington, D. C.

[36] Roe, F. I. C., Levy, L. S., and Carter, R. L.: Feeding studies on sodium cyclamate, saccharin and sucrose for carcinogenic and tumor-promoting activity. Food Cosmet. Toxicol. *8*: 135–45, 1970.

[37] Althoff, J., Cordesa, A., Pour, P., and Shubick, P.: A chronic study of artificial sweeteners in Syrian golden hamsters. Cancer Lett. *1*: 21–24, 1975.

[38] Coulston, F., McChesney, E. W., and Goldberg, L.: Long term administration of artificial sweeteners to the Rhesus monkey (M. mulatta). Food Cosmet. Toxicol. *13*: 297–300, 1975.

[39] McChesney, E. W., Coulston, F., and Benitz, K.-F.: Six year study of saccharin in Rhesus monkeys. Toxicol. Appl. Pharmacol. *41*: 164, 1977 (Abstr.).

[40] Food and Drug Administration: Histopathologic evaluation of tissues from rats following continuous dietary intake of sodium saccharin and calcium cyclamate for a maximum period of two years. Final report. Project P-169-70, Dec. 21, 1973.

[41] Tisdel, M. O., Nees, P. O., Harris, D. L., and Derse, P. H.: Long-term feeding of saccharin in rats. *In* Symposium: Sweeteners. Inglett, G. E., Ed. AVI Publishing Co., Westport, Conn., 1974.

[42] Reuben, M.: Preliminary review of carcinogenicity studies on saccharin. Unpublished, Feb. 1, 1977.

[43] Hicks, R. M., Wakefield, J., and Chowaniec, J.: Co-carcinogenic action of saccharin in the chemical induction of bladder cancer. Nature *243*: 347–49, 1973.

[44] Hicks, R. M., Wakefield, J., and Chowaniec, J.: Evaluation of a new model to detect bladder carcinogens and co-carcinogens. Results obtained with saccharin, cyclamate and cyclophosphamide. Chem.-Biol. Interactions *11*: 225–33, 1975.

[45] Ershoff, B. H., and Bajwa, G. S.: Inhibitory effect of sodium cyclamate and sodium saccharin on tumor induction by 2-acetylaminofluorene in rats. Proc. Soc. Exp. Biol. Med. *145*: 1293–97, 1974.

[46] National Cancer Institute: Guidelines for carcinogen bioassay in small rodents. PHEW (NIH) Publication 76-801, Feb., 1976.

[47] National Academy of Sciences: Principles for evaluating chemicals in the environment. Washington, D. C., 1975, p. 150.

[48] Adamson, R. H., Correa, P., and Dalgard, D. W.: Induction of tumors in nonhuman primates with various chemical carcinogens. Toxicol. Appl. Pharmacol. *29*: 93–99, 1974.

[49] Mehnert, H.: Über den relativen Wert von Zucherschtoffen and Susstoffen in der Diabetesdiat. Wiss. Veroeff. Dtsch. Ges. Ernaehr., Darmstadt, Steinkopf, 1971, vol. 20.